Appendix XVII

SUPPORTING DOCUMENT ON OVINE PULMONARY ADENOMATOSIS

1. Introduction

A type D retrovirus termed ovine pulmonary adenomatosis retrovirus (OPARV) causes a contagious disease characterised by the formation of lung tumours in sheep. The disease is known as ovine pulmonary adenomatosis (OPA) but is also referred to as jaagsiekte, ovine pulmonary carcinoma and sheep pulmonary adenocarcinoma.

The causative virus has not been cultured *in vitro* but the disease has been experimentally reproduced in lambs by infection with an OPARV cloned construct (Palmarini *et al*, 1999).

OPA closely resembles human bronchioloalveolar carcinoma (Maeda *et al*, 2001).

2. Current world situation

OPA has been reported in sheep populations throughout the world, with the exception of Australia and New Zealand (Handistatus II).

3. Clinical signs

OPA is usually a disease of adult sheep between 1 and 4 years of age. Characteristic features of the disease are dyspnea, weight loss andincreased secretion from the lungs.

4. Pathology

Even though the disease is generally referred to as an adenomatosis there is still some controversy about the pathological classification of the lung tumour as a carcinoma or adenoma (Verwoerd, 1996).

Histopathological changes due to OPARV occur in the lungs as multiple, well circumscribed nodules of transformed secretory epithelial cells, type II pneumocytes and non-ciliated bronchiolar (Clara) cells (Hunter and Munroe, 1983; Moulton, 1990). Metastases are found most commonly in the bronchial or mediasinal lymph nodes in from 10 to 50% of cases (Demartini *et al*, 1988).

Differential diagnosis

The neoplastic lesions of OPA must be differentiated from epithelial hyperplasia, a common sequel to chronic infections of sheep, such as maedi-visna or verminous pneumonia.

5. Epidemiology

Course of infection

The incubation period following experimental infection ranges from 3 weeks in lambs to several years in older animals. Tumour nodules were detected within 10-20 days following experimental inoculation of newborn lambs (Hecht *et al*, 1996). Several studies determined the peak incidence of OPA after natural infection to be 3 to 4 years (Hunter and Munro, 1983; Parker *et al*, 1998). The average duration of clinical signs is 2 months, with a range of a few days to 6 months (Moulton, 1990).

Lateral transmission

Transmission has been demonstrated naturally by aerosol and, experimentally, by contact (Demartini *et al* 1988). The disease appears to be highly contagious but susceptibility is age dependent. Lambs are more susceptible to infection if exposed prior to 10 weeks of age (Rosadio *et al*, 1988).

Vertical transmission – via embryos

OPARV has not been detected in embryos or uterine fluids. However, during the later stages of disease retroviral transcripts are widely distributed in the tissues of affected animals, including peripheral blood mononuclear cells (PBMC). Based on these findings, Parker *et al* (1998) suggested that exposure of embryos, ova and semen to virus was possible.

Vertical transmission – via semen

The distribution of retroviral transcripts could include genital tract tissues and semen could be infected. The only study conducted to investigate this found that the transfer of infection from one diseased ram to progeny and recipients did not occur (Parker *et al*, 1998). JSV has been detected in lymphoid tissues, including PBMC and dissemination of virus by these cells is thought to precede tumour formation (Holland *et al*, 1999). Any concurrent disease process in a OPARV infected ram which increases the mononuclear content of semen may lead to the production of OPARV infected semen.

Breed susceptibility

Some breeds are apparently more susceptible to disease than others (Parker *et al*, 1998; Hunter and Munro, 1983). Hecht *et al* (1996) suggest that the presence of endogenous viral sequences in sheep genomes may influence the susceptibility of sheep to the OPA.

Host range

Even though the disease has been experimentally transmitted from sheep to goats (Sharp *et al*, 1986), the lesions produced were small and circumscribed, and goats have a low susceptibility to infection (Tustin *et al*, 1988). The disease occurs naturally in goats at a very low prevalence (Tustin *et al*, 1988)

6. Adverse consequences of OPA

OPA causes significant problems in sheep in countries in which it is enzootic. OPA is recognised as economically important in South Africa, Scotland and Peru (DeMartini *et al*, 1988), but is a minor disease in Canada and USA (Radostits O.M. *et al*). among sheep causing respiratory disease followed by death in over 95% of cases. Because of the long preclinical incubation period and contagious nature of OPA as well as the short life span of most sheep under modern management systems, OPARV may enter and disseminate widely in a flock before the disease is recognised. Economic losses can arise from mortalities, the cost of control and eradication measures.

7. Risk management

a) Disease freedom of animals in country, region or flock

Country/zone freedom

Country status according to OIE can be inconsistent with reports in the literature with respect to this disease. As example, OPA was not reported in Spain or USA (Handistatus II, 1999) yet the disease has been reported in both countries in the scientific literature (Lujan *et al*, 1993; Kwang *et al*, 1995).

Flock freedom

Detection of subclinical infection is difficult and diagnosis is dependent on identifying animals in a flock with typical clinical and post mortem signs. Disease expression may not occur in flocks which do not contain animals older than 3 years. If few sheep from these flocks are necropsied and subjected to the histopathological examination required to detect early OPA the presence of the disease may not be recognised.

Infected individuals cannot be reliably detected so the infection status of individual animals depends on the disease status of the flock of origin. The observed outside median incubation period for OPA is 4 years. The observed disease free period of the flock following introduction of animals from flocks of unknown or lesser disease status must be greater than this period. This degree of caution is warranted as a number of situations could mask expression of OPA:

- the flock of origin comprises a resistant breed, or breeds,
- the flock is managed so that animals older than 3 years are culled, and
- sheep with signs of respiratory disease are culled without determination of the cause.

b) Embryo washing

Several significant trials conducted in the UK demonstrated that transmission of the virus by the transfer of washed embryos did not occur (Parker *et al*, 1998). In one study, 38 of 51 progeny from OPA positive donors survived for at least 5 years without evidence of OPA in recipients or progeny. A range of British breeds were represented in both the donor and recipient ewes. Recipients were obtained from separate flocks which had a long history of freedom from OPA. In a separate study, 4 of 5 progeny from uninfected donors mated to an infected ram survived for at least 5 years and did not develop OPA. The recipients and their progeny were kept in a closed, isolated OPA-free flock. These results have not, as yet, led to changes to the IETS (1998) categorisation and OPA is still regarded as Category 3.

c) Testing and examination

Two major difficulties hinder detection of infected sheep prior to development of clinical disease. Serum antibodies to OPARV proteins have not been definitely detected in affected sheep and most workers have concluded that a specific serological response against OPARV in naturally infected sheep and goats does not occur (York *et al*, 1992; Ortin *et al*, 1998). However, Verwoerd *et al* (1983) report the isolation of immune complexes comprising IgA and some IgG from affected lungs. An ELISA using serum has been described using a recombinant protein component of the putative virus as the antigen (Kwang *et al*, 1995).

The second difficulty arises because endogenous retroviral sequences are present within the ovine genome (Bai *et al*, 1999). These sequences within the genomic DNA of sheep hybridise to OPARV DNA probes (Hecht *et al*, 1996a; York *et al*, 1992) with the potential to interfere with nucleic acid detection methods. Fortunately, major differences between endogenous and exogenous sequences have been identified in the *env* TM and 3' unique sequence (U3) regions. Using primers in the U3 region, OPARV DNA can be detected in tumours in lung

tissue, lung secretions of infected sheep, lymphoid tissues and peripheral blood mononuclear cells (PBMC) by PCR (Palmarini *et al*, 1996, 1999). A kinetic study of OPARV infection in the mediastinal lymphocyte population of newborn lambs inoculated with the virus found that proviral DNA could be detected as early as 7 days post inoculation although the proviral burden was much less than that detected in natural cases in adult animals (Holland *et al*, 1999).

Palmarini *et al* (1995) describe the development of a blocking enzyme-linked immunosorbent assay (B-ELISA) and an immunohistochemical technique which specifically detected OPARV in transformed epithelial cells of the alveoli of OPA affected sheep. A competition radioimmunoassay (RIA) was used to detect OPARV antigen in tumour cell homogenates, lung fluid, and cell culture supernatant fluids in sheep with naturally occurring and experimentally-induced infection (Kajikawa *et al*, 1990).

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Appendix XVII (contd)

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Appendix XVIII

CHAPTER 2.4.9.

OVINE PULMONARY ADENOMATOSIS

Article 2.4.9.1.

Country or zone free from ovine pulmonary adenomatosis

A country or zone may be considered free from ovine pulmonary adenomatosis (OPA) if:

1) it has a record of regular and prompt disease reporting in all livestock;

2) no clinical, epidemiological, serological or other evidence of OPA has been found during the past 5 years;

3) OPA is notifiable in the whole country, and all clinical *cases* suggestive of OPA are subjected to field and laboratory investigations;

4) all imports of sheep (except for slaughter) from other countries or zones over the past 5 years originated from a free country, zone or flock;

5) all sheep semen and embryos/ova imported for the past 5 years fulfilled the requirements referred to in Article 2.4.9.5. and in Article 2.4.9.6., respectively.

Article 2.4.9.2.

Flock free from ovine pulmonary adenomatosis

A flock may be considered free from OPA if:

1) it is located in a country or a zone which has a record of regular and prompt disease reporting in all livestock;

2) no clinical, post-mortem, serological or other evidence of OPA has been found in any animal in the flock during the past 5 years;

3) OPA is notifiable in the whole country, and all clinical *cases* suggestive of OPA are subjected to field and laboratory investigations;

4) all sheep introduced into the flock over the past 5 years originated from a free country, zone or flock;

5) all sheep semen and embryos/ova introduced into flock for the past 5 years fulfilled the requirements referred to in Article 2.4.9.5. and in Article 2.4.9.7., respectively.

Article 2.4.9.3.

Veterinary Administrations of *importing countries* should require:

for sheep for breeding or rearing

the presentation of an *international veterinary certificate* attesting that the animals:

1) come from a country or zone free from OPA, or

2) come from an OPA free flock.

Article 2.4.9.4.

Veterinary Administrations of *importing countries* should require:

for sheep for slaughter

the presentation of an *international veterinary certificate* attesting that the animals:

1) are not being exported as part of an eradication programme;

2) showed no clinical sign of OPA on the day of shipment.

[Note: Appropriate precautions should be taken both by the exporting country *and the* importing country *to ensure that the sheep are transported directly from the* place of shipment *to the abattoir for immediate slaughter.]*

Article 2.4.9.5.

Veterinary Administrations of *importing countries* should require:

for ovine semen

the presentation of an *international veterinary certificate* attesting that:

1) the donor animals were resident since birth or for a minimum period of 5 years immediately prior to the time of semen collection in an OPA free country, zone or flock;

2) the semen was collected, processed and stored in conformity with the provisions of Appendix 3.2.2.

Article 2.4.9.6.

Veterinary Administrations of *importing countries* should require:

for ovine embryos/ova

the presentation of an *international veterinary certificate* attesting that:

1) the donor females were resident for a minimum period of 1 year immediately prior to the time of embryo collection in an OPA free country, zone or flock;

2) the embryos/ova have been collected, processed and stored in conformity with the provisions of Appendix 3.3.2.